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Abstract
Conventional treatment of thalassemia, made of regular blood transfusion and iron chelation, improves perspectives and quality of life, with more time for long-term complications to develop such as bone disease. Thalassemia bone disease (TBD) is unique: all aspects, bone anatomy, bone quality and mineral density may be affected, with important morbidity as osteoporosis, fractures, spinal deformities, nerve compression and pain. Clinical presentations include growth impairment, rickets-like features, back pain, spinal deformities, any sign from nerve compression, severe osteoporosis and fragility fractures. Age, history, physical examination and diagnostic tests support orientation on risk factors. These include bone marrow expansion, toxicity from iron overload and iron chelation, endocrine dysfunctions (hypogonadism, hypo-hyperparathyroidism, hypothyroidism, growth hormone deficiency, diabetes) and vitamin (D, C, K) and zinc deficiencies. Several of these may coexist in an individual for a long time and at different degrees, keeping a challenge to clarify the relative contribution and to select the best therapeutic options. Milestones for prevention of TBD are an early and full inhibition of bone marrow hyperplasia and iron toxicity. Empowering patient's positive resources is key for achieving on long-term healthy habits on diet, physical activity, sunlight exposure and lifestyle. Pain, related or not to bone disease, is frequent in thalassemia. The most important target for the hematologist is to prime an expert orientation on disease-related causes of pain, to drive differential diagnosis, effective pain relief and, where feasible, removal of the cause.

Learning objectives
-Participants will learn how to orient themselves among the various causes of bone disease in thalassemia and its management.
-Participants will learn how to orient themselves on disease-related causes of pain in thalassemia.

Bone disease in thalassemia

Untreated thalassemia is associated with anemia, erythroid marrow hyperplasia and skeletal deformities. Conventional treatment made of blood transfusion and iron chelation, where applied regularly, improves perspectives and quality of life. More time means also more room for long-term complications. For example osteoporosis is common in adult patients, with chronic anemia and iron overload as obvious predisposing factors, but multiple risk factors may coexist in the same patient keeping hard to quantify the relative contribution of each and to set an adequate management.
Thalassemia bone disease (TBD) is unique: all aspects, bone anatomy, bone quality and mineral density may be affected, with important morbidity as osteoporosis, fractures, spinal deformities, nerve compression and pain.[1-3]
Many factors may contribute to TBD, including bone marrow expansion, increased bone turnover, endocrine and vitamin deficiencies, toxicity from iron overload and iron chelation. In a thalassemic patient several of these factors may coexist for a long time and at different degrees. This makes difficult to clarify the relative contribution to TBD and to select the best therapeutic options.

Factors predisposing to TBD

Bone marrow expansion
Abnormal proliferation of bone marrow cells, independent of hematopoietic lineage, is associated with bone loss.[1] In severe thalassemia, ineffective erythropoiesis causes a bone marrow expansion by a factor of up to 30 times, that is not fully cancelled even with an optimal transfusion regimen. Medullary trabeculae are destroyed with cortical thinning.[4] The skull may have a "hair-on-end" appearance with bossing of facial bones, malocclusion and the typical thalassemic facies. Long bones, mainly humeri, may loose the concave profile and be short, with signs of growth arrest and

recovery like transverse epiphyseal radio-dense lines.

Iron overload

Iron metabolism may independently contribute to bone homeostasis.[1] Reduced hepcidin levels negatively affects bone homeostasis by lowering bone formation and increasing bone resorption.[5] In vitro studies demonstrate that iron inhibits human osteoblastic activity and favors osteoclast differentiation and bone resorption,[6] through elevated RANKL/OPG ratio.[7] In humans with hemochromatosis or secondary iron overload, the causative relationship with osteoporosis is clouded by the co-presence of hypogonadism and other endocrinopathies, but a strong trend to osteoporosis and fractures are documented also in young eugonadal patients, as well as improvement with iron clearing.[8]

Some findings indicate that in thalassemia intermedia and thalassemia major bone disease differs at least in one aspect, low versus high bone turnover respectively, with implications for treatment.[9] The origin of such a difference may reside in different levels of iron overload and iron turnover.

Iron chelation

A few studies specifically addressed the role of iron chelation therapy in the prevention or control of iron-overload-associated bone disease. Both positive and negative effects of iron chelation have been reported.

Deferoxamine has been associated with bone dysplasia, especially with early start, high doses and reduced iron stores. Long bones show metaphyseal changes with rickets-like widening and sclerotic lesions. Severe epiphyseal dysplasia may cause genu varum or valgum and require surgery. Vertebral body changes, mainly platyspondyly at the thoracolumbar tract, result in a short and kyphotic trunk.[10]

These changes have not been observed with the oral chelators. Deferiprone may induce arthropathy with synovial and cartilage alterations and sub-chondral bone flattening. Deferasirox has been associated both with an increase in BMD,[11,12] and with hypercalciuria and nephrocalcinosis. [13,14]

Hypogonadism

Free estrogen and testosterone are key in increasing OPG mRNA and decreasing RANKL. Lack of sex steroids with delayed puberty from any cause contribute to failure of achieving optimal peak bone mass and predisposing to severe osteoporosis.[15] In thalassemia early pituitary damage from iron toxicity is responsible of hypogonadotrophic hypogonadism and delayed/incomplete puberty. This condition is very common even today in patients treated according to the best standards.[16]

Parathyroid dysfunction

Hypoparathyroidism is a late but common complication of iron overload/toxicity with poor or no chelation. While its relationship with hypercalciuria and nephrolithiasis is clear, it is not so for osteoporosis, with contrasting findings in different papers. In optimally treated patients hypoparathyroidism is uncommon, whereas secondary hyperparathyroidism due to vitamin D deficiency is receiving more attention, with increasing diagnostic challenges in the individual patient.[3,17]

Hypothyroidism

Both hyperthyroidism and hypothyroidism are associated with osteoporosis and increased risk of fractures.[18] In thalassemia hypothyroidism is frequent and easily corrected with thyroid hormone replacement. On the other side the time on hormone replacement is inversely related with BMD and increases the risk of fractures even in the presence of euthyroidism. This must suggest caution in prescribing long-term thyroxin treatment in any thalassemic patient with borderline thyroid function.

Growth Hormone (GH) deficiency

During childhood and adolescence GH plays a key role in linear growth and attainment of appropriate height and peak bone mass. GH interacts directly with GH receptors on osteoblasts and via locally produced IGF1. GH deficiency with low IGF-I and the corresponding binding protein is frequent both in pediatric and adult patients and has been related with iron toxicity.[19] Treatment with GH increases vertebral and femoral BMD over time.[20]

Diabetes

Insulin-dependent diabetes type 2 in thalassemia is a long-term complication from iron toxicity on pancreatic cells. It is more frequent in adult patients with poor control of iron overload.

Liver disease

Hepatitis C virus is now disappearing where direct-acting antiviral agents have been applied,[21] but a long exposure to viral and iron load may make chronic liver disease and cirrhosis irreversible. This may favors bone disease throughout different pathogenic mediators, like fibronectin, insulin like growth factor-I, and vitamin D metabolism.

Renal disease

Differently from the past, kidney dysfunction has a growing importance in thalassemia, due to prolonged survival and

raising frequency of renal hyperfiltration, hypercalciuria, kidney stones and tubular dysfunction.[22]

Vitamin D deficiency

The prevalence of vitamin D deficiency is high in thalassemic patients, even in high sunshine exposure regions and normal diet. The diagnosis of deficiency is elusive. Signs and symptoms of vitamin D deficiency are nonspecific and common in thalassemia: muscle weakness, bone pain, osteopenia/osteoporosis. The presence of radiological and MRI changes in the long-bone metaphyses, periosteal reaction and adjacent soft-tissue edema have diagnostic value, but their absence does not exclude vitamin D deficiency. The assessment of 25-hydroxy vitamin D serum levels is key for vitamin D deficiency, but may overestimate it, due to pre-analytical (season, skin color) and analytical (different assays variability) reasons.[23]

Secondary hyperparathyroidism is strongly associated to vitamin D deficiency. These two conditions, throughout calcium channels regulation, may be the bridge to cardiac iron load and dysfunction.

Vitamin C deficiency

In subjects with a normal diet, vitamin C deficiency is possible in the presence of hyper-consumption, like chronic iron toxicity.[24] In thalassemic patients with high iron overload and poor or no chelation, ascorbate deficiency may contribute to TBD, by impairing chondrocytes and osteoblasts function, with impaired long bones growth, subperiosteal hemorrhage and fractures.[25]

Vitamin K deficiency

Even though the effect of vitamin K2 on bone mass density is limited, its deficiency may contribute to osteoporotic fractures, whereas vitamin K2 administration improves osteoblastic function and inhibits osteoclastic one. In thalassemia vitamin K2, combined with calcitriol, improves lumbar spine BMD.[26]

Zinc deficiency

Many other deficiencies that may potentially impact on bone health and growth in thalassemia have been reported, but for a few of them a clinical relevance has been demonstrated.

Low serum zinc levels are frequently observed in thalassemia and associated with hemolysis, oxidative damage and the effect of iron chelators.[27] Benefits for osteoporosis and height growth are based on uncontrolled studies.[28]

Table: Schematic summary of factors that may contribute to thalassemia bone disease (TBD) and their management.

Factor	Key mechanism	Treatment	Prevention
Bone marrow expansion	Ineffective erythropoiesis	Transfusion at optimal Hb levels	Transfusion at optimal Hb levels
Iron overload	Iron toxicity	Optimal iron chelation	Early and regular iron chelation
Iron chelation	Over-chelation; drug toxicity	Tune chelation intensity on iron overload; avoid high doses	Tune chelation intensity on iron overload
Hypogonadism	Iron toxicity	Replacement therapy	Early and regular iron chelation
Hypoparathyroidism	Iron toxicity	Replacement therapy	Early and regular iron chelation
Hyperparathyroidism	Vitamin D deficiency	Vitamin D2 or D3	Vitamin D2 or D3
Hypothyroidism	Iron toxicity	Replacement therapy	Early and regular iron chelation
Growth Hormone deficiency	Iron toxicity	Replacement therapy	Early and regular iron chelation
Diabetes	Iron toxicity	Replacement therapy	Regular iron chelation; lifestyle
Liver disease	Viral hepatitis; Iron toxicity	Antiviral therapy; regular iron chelation	Safe blood; regular iron chelation
Vitamin D deficiency	Iron toxicity	Vitamin D2 or D3	?
Renal disease	Hypercalciuria	Correct causes	?
Vitamin C deficiency	Iron toxicity	Vitamin C rich diet	Optimal iron chelation

Clinical aspects and management of TBD

Bone disease in thalassemia may be asymptomatic for years. The starting point in the individual patient is to assess the relative contribution of the many potential risk factors. Age, history, physical examination and diagnostic tests are helpful in orienting the diagnosis. Clinical presentations include growth impairment, rickets-like features, back pain, spinal deformities, any sign from nerve compression, severe osteoporosis and fragility fractures. Often osteoporosis-related fractures, including vertebral ones are unappreciated. In adults height loss from serial height measurements is a useful tool in detecting patients with vertebral fractures and lowering future fracture risk.[29]

In very young patient any sign of *bone marrow expansion* and bone enlargement must be assessed and followed. In the absence of any validated method to quantify them, changes at serial checks are useful in predicting progression. Imaging findings on radiography and MRI studies and X-rays are useful, as well as serial pictures of the patient and even at diagnosis asking parents to share previous pictures may help.
The start of regular transfusion is able to freeze or even reverse bone expansion. Mean pre-transfusional hemoglobin levels must be adequate (9-9.5 g/L in beta thalassemia major). Full reversal is possible only during the first years of life. Plastic surgery is rarely applied to thalassemia, whereas orthodontic interventions are effective but require light forces and special care.[30]

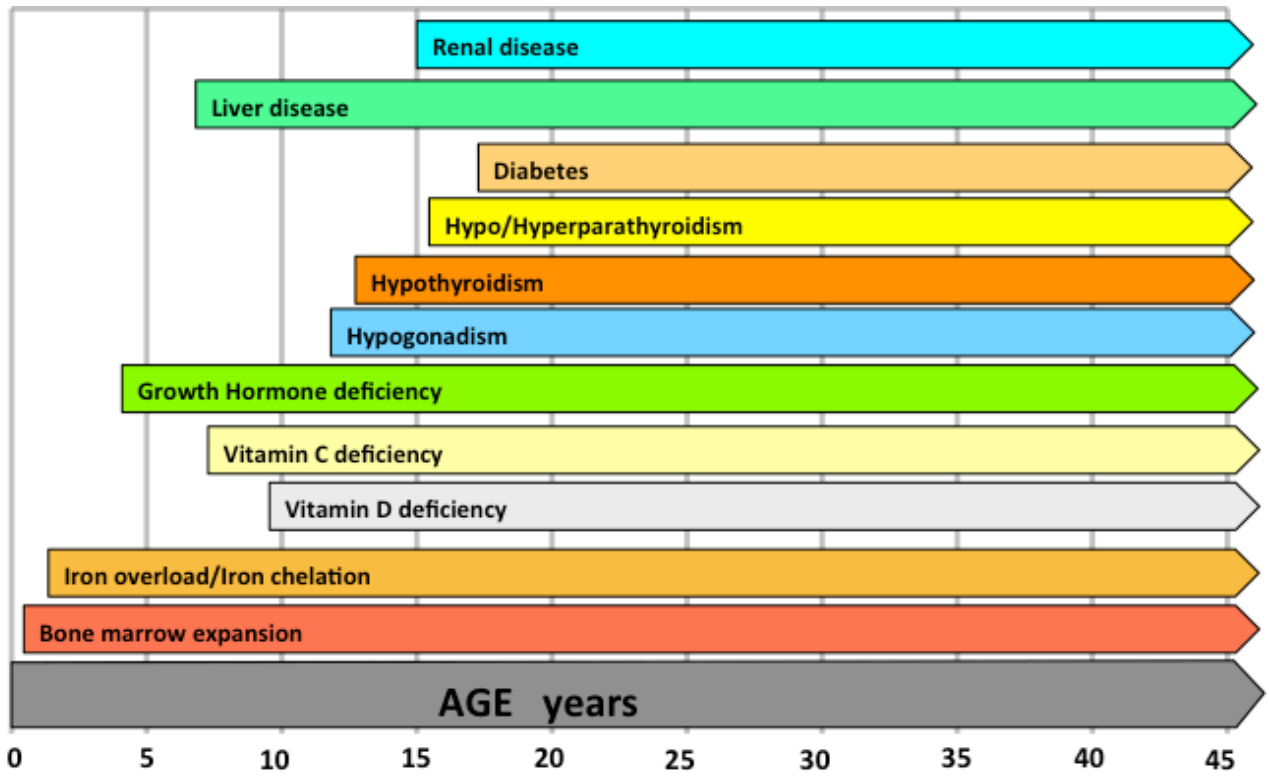
Reducing a severe *iron overload* must always be a priority in treating or preventing TBD, due to the deleterious effect of iron toxicity.[6] Choice of chelators, doses and *iron chelation* scheme should be tailored to fit individual needs. Strict monitoring may prevent overchelation that, at least for deferoxamine, is associated with bone alterations.[10]

Hypogonadotrophic *hypogonadism* and *GH deficiency* remain frequent even in the modern era of iron chelation, as the cause is an early and irreversible damage from iron toxicity to sensitive pituitary cells. It is difficult to prevent this toxicity, at least in thalassemia major, as it is difficult to handle iron chelation in infanthood at low iron overload. The impact of *hypogonadism* and *GH deficiency* on TBD is very important, as both hinder during childhood and puberty the progress to a normal peak bone mass, severely predisposing to osteoporosis in adulthood. The diagnosis of *hypogonadism* is clinical, based on the absence of pubertal development or delay or incomplete maturation of secondary sex characteristics, confirmed by low LH, FSH and estradiol/testosterone levels.
To prevent *hypogonadism* and *GH deficiency*, *iron chelation* should start as early as possible. It is likely that the recommendation of most guidelines, to start iron chelation after 10-20 transfusions or when serum ferritin is above 1000, is inadequate.[31]
Throughout childhood and adolescence, biannual growth assessment, including standing and sitting height, bone age and pubertal staging, is functional to well-timed diagnosis and treatment. In deficient patients, replacement therapy with GH or sexual hormones has been proven effective in thalassemia.[31] Zinc supplementation may be considered in individual patients with low serum levels.
The decision to start supplementing and prevent *vitamin D deficiency* is based on regular monitoring of serum level of 25-OH vitamin D. A calcium-rich diet and cholecalciferol should be preferred to oral calcium and active metabolites in a condition like thalassemia, where independent factors contribute to hypercalciuria and the risk of kidney stones and nephrocalcinosis. The presence of *hypoparathyroidism* requires a more aggressive approach.[32]

Management of osteoporosis in thalassemia follows general principles. Treatment should always be considered if osteoporosis is present, there is a history of fragility fracture, or in the setting of osteopenia plus high risk for fracture. There is evidence that bisphosphonates in thalassemia improve bone mineral density but data on long-term effects are lacking.[32] Oral alendronate, intravenous zolendronate, neridronate and clodronate have been tested in randomized controlled trials. Even if head to head trials have not been done, zoledronate and neridronate should be considered as first-line agents in the management of thalassemia-associated osteoporosis.[32] Prescribers should be aware that also in thalassemia side effects like atypical fractures of the femur have been found.[32,33]
Other interventions for TBD, such as denosumab, strontium ranelate and teriparatide, have been published. All of them are interesting for the mechanism of action, but cannot be recommended due to the limited data available.[16,32]

Prevention of TBD

In any severe thalassemia phenotype, milestones for prevention of TBD are an early and full inhibition of bone marrow hyperplasia and iron toxicity. This may be accomplished by early start of regular transfusion and iron chelation. Excluding specific circumstances, to leave unbalanced severe anemia and iron overload and prescribe drugs for TBD should be considered a medical nonsense and raise ethical concerns. An approximate timeline of the occurrence of complications or conditions relevant for bone disease if the treatment of thalassemia is not optimal is shown in the figure.



Each thalassemic patient, regardless transfusion-dependence, should receive full information about risk factors and early signs/symptoms of TBD. Follow up should be personalized according to age and risk factors. Treating staff should be motivated in empowering patient's positive resources, as the only key for achieving on long-term healthy habits on diet, physical activity, sunlight exposure and lifestyle.

Pain in Thalassemia

The most important target for the hematologist or any medical professional is to outline an expert orientation on disease-related causes of pain, to drive differential diagnosis, effective pain relief and, where feasible, removal of the cause.

Differently from sickle cell disease, pain is uncommon during the first years of life and becomes frequent in adult life.[34] Most of patients more than 35 years old refer chronic pain of moderate-severe intensity. Pain severity increases with age, but does not vary significantly with sex or thalassemia diagnosis. Quality of life due to pain in thalassemia declines greatly with age, compared with the general population.[35] Chronic pain is more frequent in patients with late start of regular transfusions and a diagnosis of thalassemia intermedia, and is associated with a more expanded hypercellular bone marrow on MRI.[36] Hydroxyurea treatment is able to induce MRI modifications and pain relief.[36]
The most common site of chronic pain is lower back. Pain may be triggered by physical activity like prolonged standing and lifting heavy objects, but the most frequent pain trigger is low hemoglobin level, with relief from transfusion, especially in patients with longer transfusion cycles.[35,37,38] MRI imaging of spine may reveal abnormal vertebral morphology, disc degeneration and various degree of osteoporosis. Changes are more extensive than in patients with back pain and no thalassemia.[39] Collapse or crush fractures of the vertebral body are also frequently seen.
Patients with more sites of pain or more visits with pain showed higher symptoms of depression and anxiety.[40]

Diagnostic orientation by site

Headache
Headache characteristics like onset, intensity, recurrence and the coexistence of fever or neurological signs help orientation. Disease-related causes of headache include infections, mainly in patients with severe iron overload and splenectomized: cerebral abscess, meningitis. Otitis and sinusitis are more frequent in patients with thalassemic bone alterations. Occurrence of extramedullary erythropoiesis is possible but uncommon.

Chest pain
Onset, localization and breath involvement help diagnostic orientation. In under-treated patients rib displacement or fracture and osteochondritis are common. Splenectomized patients have a raised risk of pulmonary embolism. Onset of congestive heart failure may be slow and hidden with no edemas, mild weight gain and isolated pain due to liver capsule distension; depending on irradiation pain may involve the abdomen or back or chest. Aseptic pericarditis should be considered in case when severe iron overload goes along with no chelation. Acute coronary syndrome is uncommon in thalassemia. Masses of extramedullary erythropoiesis are typically asymptomatic, remain undiagnosed and do not cause pain if not for nerve compression.

Abdominal pain
Onset and localization help diagnostic orientation. Gallstones, cholecystitis, cholangitis and pancreatitis must always be taken into account. Kidney stones are frequent in thalassemia due to hypercalciuria. In patients on deferoxamine pain may be the first sign of a life-threatening Yersinia infection. As for chest pain, abdominal pain may flag the onset of congestive heart failure. Splenectomized patients are at risk of portal vein thrombosis. Back or abdominal pain may also indicate a delayed hemolytic transfusion reaction. Masses of extramedullary erythropoiesis are typically asymptomatic, remain undiagnosed.[41]

Back pain
Osteoporosis may pass asymptomatic or cause compression signs and pain due to micro-fractures and vertebral bodies flattening.[16] The same may happen for disk degeneration or for the presence of extramedullary erythropoiesis.
Kidney stones are frequent in thalassemia due to hypercalciuria. Also gallstones with posterior irradiation should be considered in case of back pain. Back pain, due to liver capsule distension, may herald a congestive heart failure.
Back or abdominal pain may also indicate a delayed hemolytic transfusion reaction.

Foot pain
Foot stress fractures are associated with osteoporosis and TBD.

Joints pain
In thalassemia arthropathy or arthritis may be associated to distinct factors: iron overload in the absence of iron

chelation, hyperuricemia and deferiprone.[42]

Conclusions

Conventional treatment of thalassemia improves perspectives of life, with more room for long-term complications such as bone disease and pain. A better knowledge of their many causes is key for optimal management and prevention.

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